Kasetsart J. (Nat. Sci.) 49 : 413 - 421 (2015)

Biology and Demography of a Springtail, *Xenylla* sp., Reared on a Substrate Treated With Effective Microorganism (EM)

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ABSTRACT

Effective Microorganism (EM) is a proprietary liquid containing many co-existing microorganisms and is widely used to increase crop yields in Thailand. However, there is little knowledge and no scientific proof on the influence of EM on soil microarthropods which serve as bioindicators of soil fertility. There is a possibility that EM promotes their growth and indirectly improves the soil fertility. EM was added to the substrate and the biology of *Xenylla* sp. was compared to substrate treated with molasses and a control added with water only. Observations under a stereo microscope were made to record the life history and a life table was constructed. EM caused a significantly lower hatching percentage and adult life expectancy and the developmental time from the juvenile to adult stage was delayed when compared to the control, but not to the molasses ($P < 0.05$). However, the effects on net reproductive rate, finite capacity of increase, intrinsic rate of natural increase, generation time and doubling time were not significant, probably due to the rapid growth of fungi that covered the egg surfaces causing water loss and lower egg viability. The lower relative humidity and accumulation of metabolic wastes by fungi are believed to suppress the well being of the insects. EM did not demonstrate significantly positive effects on the biological performance of this *Xenylla* species.

Keywords: collembolan, Hypogastruridae, Hexapoda, life table

INTRODUCTION

Effective Microorganism (EM), a liquid containing many co-existing microorganisms was developed by Teruo Higa of Ryukyus, University in Japan in the 1980s. The application of EM is considered a natural practice in agriculture since no chemicals are used and it is marketed as a commercial product by authorized licensees around the world. The major groups of microorganisms in EM are photosynthetic bacteria, lactic acid bacteria, yeasts, actinomycetes and fungi. As a result, the added microbial organisms are expected to help improve the soil conditions, suppress pathogenic microorganisms and increase the efficiency of organic matter utilization by plants. Users can purchase stock of EM and produce their own formula to suit their own purpose or they can purchase ready-made stock of their choice. In Thailand, EM was introduced in 1989 and has been applied in agricultural soil by mixing with plant-extracted substances such as chili pepper, mint and grass or by mixing with vinegar and rice whiskey (Higa, 1999). Public acceptance of and satisfaction with the products are generally on the positive side, possibly based on better crop performance (O *et al*., 2008; Wood *et al*., 2008; Yadav, 2008).

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Received date : 02/09/14 Accepted date : 15/01/15

Even though a number of studies have been undertaken to assess the impacts of EM on agriculture, there has been no scientific proof of its efficiency and mechanisms. It is of interest to know whether EM promotes the population growth of beneficial soil fauna and, in turn, helps increase the organic matter and improve soil quality. Better soil quality might lead to an improvement in plant characteristics such as higher yield, increased growth rate and resistance to pests. One group of animals which deserves such attention is the springtails which are generally believed to be a bio-indicator of soil fertility. To date, most work with springtails in an agricultural environment has been on the impacts of insecticides and mineral concentrations.

Springtails are minute, wingless hexapods that often have a jumping apparatus called the furcula located on the abdomen and belong to the order Collembola (Hopkin, 1997). Most species live in soil, leaf litter and refuse with high humidity and in nature, springtails are among the most numerous of forest soil invertebrates, contributing along with other animals to the decomposition of litter and soil formation through their feeding activities (Swift *et al*., 1979). According to the evidence from gut analysis of springtails, fungal hyphae were found to constitute a large fraction of the diet of many collembolan species (Newell, 1984; Moore *et al*., 1987). Since fungi are usually added in EM, there is the possibility that an increase in the fungal population could provide more nutrients for springtails. This could lead to an increase in the population of springtails. The decomposition rate could be increased to produce more organic matter in the soil.

Based on the information from local farmers and from the recommendations by some authorities on the use of EM to improve crop performance, the aim of the current study was to evaluate the influence of EM on the biology and demography of a springtail species in the genus *Xenylla* (Family Hypogastruridae) under laboratory conditions. This species was chosen because it is generally found in high abundance in many soil types in Thailand. It has been successfully mass-cultured in the laboratory used for this study and has been maintained as stock culture for 8 yr. Descriptions of the species will be reported separately and there is a possibility of a new species.

MATERIALS AND METHODS

Preparation of EM solution

A commercial formulation of EM was prepared according to the labeled instructions from the stock solution of EM by the trade name of EX-M. The EM stock contains EX-M stock, molasses and water in the ratio 1:1:20 by volume, respectively. When used in the current experiments, the EM stock was diluted to 1×10^{-3} according to the formulation used by farmers. Since molasses was one of the components in EM, it was also used in the tests.

Springtail culture

Originally, a species of springtail in the genus *Xenylla* was collected from soil on the Salaya campus, Mahidol University, Nakhon Pathom province, Thailand. Individuals were extracted alive using a Berlese-Tullgren funnel. Bottles 4.5 cm in diameter and 6 cm tall with a base of plaster of Paris-charcoal substrate (a mixture of plaster-charcoal and distilled water in the ratio 8:4:5.5 by volume, respectively) were used to maintain stock cultures at 25–28 °C. Baker's yeasts were given as food. In order to keep the culture bottles moist, water was added in drops every 3 d depending upon the dryness of the culture medium. The stock culture has been maintained in the soil microarthropod laboratory at the Department of Biology, Faculty of Science , Mahidol University, Bangkok since 2005 and has been and continues to be used in various research studies as well as for teaching purposes.

Effects of EM on biology of *Xenylla* sp.

Comparisons were made among the springtails reared with EM, molasses and the control (water added). Molasses was prepared in solution by dissolving in water in the ratio 1:1,000 by volume.

Effects on egg stage

In total, 30 one-day-old eggs were placed in a Petri dish (9 cm diameter) with a base of plaster of Paris-charcoal substrate. The Petri dishes were treated with EM or molasses and water was used as the control. The number of juveniles hatched was recorded daily for 2 wk until it was clear that no more eggs would hatch. Each treatment was repeated five times.

Effects on juvenile stages

In total, 90 one-day-old juveniles were transferred using a fine soft brush to the bottles that were treated with water (as the control), EM or molasses with one individual per bottle, making 30 bottles for each treatment. The juveniles were observed daily and the numbers of dead bodies and exuviae were recorded until they reached the sixth juvenile stage. During the experiments, baker's yeasts were given as food every 3 d and the solutions were supplied once a week.

Effects on adult stage

A sample of 100 one-day-old eggs was transferred using a fine soft brush into 10 bottles with a base of the plaster of Paris-charcoal substrate. After hatching, the juveniles were transferred to new bottles (1.8 cm in diameter and 4.7 cm tall) with 10 individuals per bottle. Similar treatments were prepared using EM or molasses added to the substrate. Juveniles were left to develop until the adult stage was reached. During the experiments, baker's yeasts were given as food every 3 d and the solutions were supplied once a week. Observations on the developmental stages and fecundity were made under a stereo microscope. The data were used to construct life tables.

Estimation of life table statistics

The life tables were constructed based on the observed data of the *Xenylla* cultured in substrates added with EM, molasses or water. The following parameters (Neal, 2004) were calculated.

The mean life expectancy (e_x) is the estimated length of time that an average animal is expected to live and was calculated using Equation 1:

$$e_x = T_x / l_x \quad (1)$$

where l_x and T_x are, respectively, the age-specific survival rate and sum of the number of survivors in age (x) and older age classes.

The intrinsic rate of natural increase (r_m) was estimated from the data using Lotka's Equation 2:

$$1 = \sum_0^\infty e^{-r_m x} l_x m_x \quad (2)$$

where l_x, m_x and x are, respectively, the age-specific survival rate, the age-specific fecundity and age (in days).

The net reproductive rate (R_0) is the number of female offspring produced per female and was calculated using Equation 3:

$$R_0 = \sum l_x m_x \quad (3)$$

where l_x and m_x and x are, respectively, the age-specific survival rate and the age-specific fecundity.

The finite capacity of increase (λ) is the number of times that the population will multiply itself per unit of time and was calculated using Equation 4:

$$\lambda = e^{r_m} \quad (4)$$

The generation time (T) is the time between the birth of a parent and the birth of its offspring and was calculated using Equation 5:

$$T = \ln R_0 / r_m \quad (5)$$

The doubling time (D) is the number of unit time (in days) required for the population to double its number and was calculated using Equation 6:

$$D = \ln 2 / r_m \quad (6)$$

Data analysis

Data were analyzed using a single factor analysis of variance (ANOVA) to detect the effects of EM on the biology of *Xenylla*. Tukey's honestly significant difference was used for the comparison of means. Data were log-transformed prior to the analyses to improve homogeneity of variances, if necessary. Nonparametric data were analyzed using the Kruskal-Wallis test and the Mann-Whitney test was applied to detect any significant differences between treatments. Differences among groups were considered significant at $P < 0.05$.

RESULTS

Effects on egg stage

The parameter egg viability was expressed as the percentage of hatching. The number of juveniles born was counted daily for 2 wk. Figure 1 demonstrates that EM had negative effects on the percentage of hatching of *Xenylla* eggs. The eggs laid on the substrate treated with EM hatched at an average of 75.33 ± 4.42% which was significantly lower than for the control (93.33 ± 3.80%) (F = 5.404; degrees of freedom (df) = 2, 12; P = 0.021) whereas the hatching of eggs on the substrate treated with molasses was not significantly different from the control and EM ($P > 0.05$).

Effects on juvenile stages

The developmental time (from hatching to the end of the last juvenile stage) was recorded (Table 1). It was found that the time spent from one juvenile stage to the next was not significantly different in all treatments, but the overall period from the birth of the first juvenile to the last day of the juvenile stage was different as the juveniles exposed to EM required significantly longer to emerge as adults (16.08 ± 0.199 d) than the control but was not significantly different from those on the molasses (15.43 ± 0.189 d) (F = 3.225; df = 2,

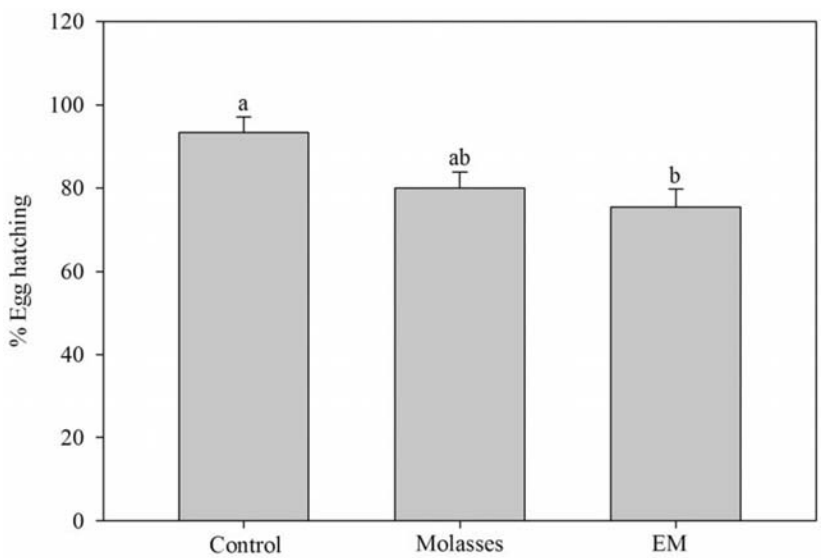

Figure 1 Mean percentage of egg hatching of *Xenylla* sp. treated with water (control), molasses and Effective Microorganism (EM). The same letters above a bar indicate no significant difference ($P > 0.05$).The symbol (┬) indicates the SE.

242; $P = 0.041$). For those exposed to molasses, the time was not significantly different from the control ($P > 0.05$).

Effects on adult stage

The average longevity and fecundity of adults were calculated. The fecundity of springtails was represented as the total number of eggs laid by a female throughout her life. The averages of the total number of eggs were not significantly different among springtails in all treatments as shown in Table 2. Based on the estimation of life table statistics, EM and molasses both caused a significant decrease in the life expectancy (e_x) when compared to the control ($F = 7.023$, df = 2, $P = 0.27$), while all other parameters did not demonstrate any significant differences among the control, molasses and EM. *Xenylla* sp. was expected to live longest (45.18 ± 1.84 d) on the control compared to 39.63 ± 1.08 d and 39.92 ± 0.801 d on molasses and EM, respectively. However, the life expectancy of *Xenylla* sp. reared on either substrate with molasses or substrate with EM was not significantly different.

Table 1 Mean developmental time of *Xenylla* sp. juveniles on substrate treated with water (control), molasses and EM (n = 90)

Juvenile stage	Mean ± SE (d)		
	Control	Molasses	EM
I–II	4.45 ± 0.091[a]	4.42 ± 0.106[a]	4.41 ± 0.081[a]
II–III	2.14 ± 0.058[a]	2.13 ± 0.044[a]	2.15 ± 0.052[a]
III–IV	2.10 ± 0.052[a]	2.25 ± 0.053[a]	2.26 ± 0.064[a]
IV–V	2.21 ± 0.063[a]	2.15 ± 0.046[a]	2.35 ± 0.069[a]
V–VI	2.28 ± 0.077[a]	2.45 ± 0.080[a]	2.46 ± 0.086[a]
VI–Adult	2.26 ± 0.083[a]	2.28 ± 0.092[a]	2.45 ± 0.117[a]
Total	15.43 ± 0.189[a]	15.70 ± 0.164[ab]	16.08 ± 0.199[b]

EM = Effective Microorganism.

Data were analyzed using one-way ANOVA ($P < 0.05$). Values with the same superscript lowercase letter within a row are not significantly different at the 5% level by the Tukey test.

Table 2 Life table statistics of *Xenylla* sp. treated with water (control), molasses and EM (n = 15)

Parameter	Mean ± SE		
	Control	Molasses	EM
*R_0	17.08 ± 5.29[a]	25.23 ± 6.33[a]	18.62 ± 3.42[a]
λ	1.085 ± 0.005[a]	1.096 ± 0.008[a]	1.088 ± 0.005[a]
r_m	0.082 ± 0.005[a]	0.092 ± 0.008[a]	0.084 ± 0.005[a]
e_x	45.81 ± 1.84[a]	39.63 ± 1.08[b]	39.92 ± 0.801[b]
T	33.52 ± 1.47[a]	34.16 ± 0.54[a]	34.46 ± 0.34[a]
D	8.54 ± 0.48[a]	7.64 ± 0.67[a]	8.31 ± 0.30[a]

EM = Effective Microorganism; e_x, = Mean life expectancy; r_m = Intrinsic rate of natural increase; R_0 = Net reproductive rate; λ = Finite capacity of increase; T = Generation time; and D = Doubling time.

Data were analyzed using One-way ANOVA ($P < 0.05$). Values with the same superscript lowercase letter within a row are not significantly different at the 5% level using the Tukey test.

* = Data analyzed using the Kruskal-Wallis test ($P < 0.05$).

DISCUSSION

It was hypothesized that the application of Effective Microorganism (EM) in agricultural soil helps improve the crop yields through the feeding of soil insects such as springtails. EM might have a significant role in promoting indirectly the growth or development of springtails (*Xenylla* sp. in this case) due to the presence of the microorganisms including fungi in the soil for *Xenylla* to feed on and result in a higher content of organic matter. Soils are also inhabited by a large number of beneficial microarthropods, and soil insects such as springtails are one of the major groups in most soil types (Swift *et al*., 1979). Adding EM to the soil would probably enhance the performance of those insects since they are the primary consumers of organic materials and fungal feeders. With an increasing amount of fungal food, springtails could have a larger population and be more effective in the turnover of organic matter. This would result in enhanced soil fertility and crop yields would increase. Evidence has been reported that due to the nitrogen content in their hyphae, feeding favorable fungi had positive effects on the growth rate and fecundity of some springtails (Booth and Anderson, 1979; Chen *et al*., 1995; Klironomos *et al*., 1999)

The current experiments were designed to record the biological performance of springtails exposed to EM, compared with the control (water) or with molasses which is a component added to EM solution in commercial use. Parameters determined were the egg viability in terms of hatching percentage, the developmental time from the juvenile to adult stage and the life table statistics of adults. In the experiment, 1 cm^3 of EM was added to the substrate before the start and 0.15 cm^3 was added once a week until the end of the experiment. This followed the practice of the majority of farmers in Thailand. When EM was sprayed into the bottle, it was taken up by *Xenylla* sp. individuals through two possible routes: either passing into their bodies by the ventral tube (the collophore) which is associated with water and salt exchange (Hopkin, 1997) or through the indirect absorption of EM by way of feeding on baker's yeasts.

The results do not support the hypothesis. Instead, EM even acted negatively in some aspects under laboratory conditions. The percentage of egg hatching treated with EM was lower and the developmental time from the juvenile to adult stage was longer than for the control. It appears that EM as well as molasses did not have any effect on the reproduction (R_0, λ, and r_m) and *Xenylla* sp. was even expected to have a shorter life on molasses or EM than on the control. However, unlike EM, molasses had no effect on the egg and juvenile stages of *Xenylla* sp. perhaps because in the treatment at the egg stage, a rapid growth of fungi was found to cover the surfaces of eggs which led to the lower rate of egg hatching. When EM was added into the substrate, it helped increase the number of microorganisms (including fungi) resulting in unsuitable conditions for eggs. In addition, fungal hyphae that covered the eggs might absorb water and nutrients from eggs, resulting in the inhibition of egg hatching.

The delayed developmental time of the juvenile stages is not clearly understood. However, it might have been due to the decrease in humidity and the accumulation of waste products from microorganisms added into the culture bottles. EM and springtails might take up a large amount of water for their metabolism. The relative humidity (RH) was reported to have effects on various species of springtails in several works. For example, five epigeonistic species of springtails required 100% RH for survival (Davies, 1928) and *Onychiurus armatus* required 100% RH for its long-term survival (Mayer, 1957). Euedaphic springtails were also reported to be very susceptible to low humidity (Ashraf, 1971). In addition, both temperature and humidity were demonstrated to play an important role in embryonic and post-embryonic development of springtails in the family Hypogastruridae and the

highest post-embryonic survival of the species investigated was from 98 to 100% RH (Thibaud, 1968a, 1968b, 1970).

Water is essential not only for the activities of soil microorganisms, but also for microbial activity (Sylvia *et al*., 2005). When EM was added into the bottles, it did not only compete for water to be used in metabolic activity, but it also caused a decrease in humidity in the bottle which in turn delayed molting of juveniles in each stage, hence leading to a longer total developmental time of *Xenylla* sp. from juvenile stage I to the adult stage when exposed to EM.

In terms of waste products excreted from microorganisms, although the substrate had charcoal that absorbed any harmful volatiles from the plaster or metabolites from the cultured animals (Booth, 1983), it had limited absorption capacity due to the surface area of the charcoal substrate and the quality of the charcoal. EM added into the bottles may have been the cause of a higher rate of metabolic waste production than of absorption. The waste buildup finally had negative effects on the growth of animals. This result was in agreement with the observation of springtails in the culture stock, where the rate of molting and oviposition of springtails reared on old substrate was usually lower than those reared on the fresh culture substrate (data not shown). Booth (1983) reported that the higher the proportion of charcoal in the culture substrate, the higher the molting rate, egg laying rate and clutch size of eggs of the springtail, *Folsomia candida*. This indicates the importance of waste products in the substrate on the growth and fecundity of springtails.

In the adult stage, the life expectancies of *Xenylla* sp. treated with EM and molasses were significantly shorter than for the control ($P < 0.05$) whereas other parameters were not significantly different. The only factor that may be responsible is the growth of fungi in the substrate. A mixture of different fungi was present in EM. Molasses was also reported to be a carbon source for the growth of many fungi (Sughra *et al.*, 2013). *Xenylla* sp. was reported to be somewhat specific in its requirements for some kinds of food in order to grow well. Preferred foods, especially a fungal diet, generally resulted in higher egg production and survival (Addison and Parkinson, 1978; Klironomos *et al*., 1995; Chen *et al*., 1999). Other studies reported that the food quality varied with fungal species and that the quality of food is an important driving factor for population dynamics in springtails (Joosse and Testerink, 1977; Walsh and Bolger, 1990; Chen *et al*., 1995). However, from the current observations, fungi grew well and very fast in the bottles and a number of adults were frequently trapped in the hyphae. In closed containers, instead of being beneficial, too many fungi become harmful and affected the well being of the adult springtails. It is clear from this study that laboratory conditions brought about some problems that were unlikely to happen in a field environment. The main problem was the fungi that covered the surface of the eggs, as in nature, these fungi would be consumed by other springtail juveniles or adults, allowing the eggs to hatch. When the effects of EM on the eggs were investigated in the current study, the eggs were isolated in the bottles in the absence of active juvenile and adults, resulting in the buildup of fungi which eventually dried up the eggs.

Based on overall performance, EM did not demonstrate possible positive effects on *Xenylla* sp. studied under laboratory conditions. The hypothesis is recommended for further tests using natural or artificial soil or under field conditions. Field studies might result in different outcomes due to other environmental factors, that is, predators, soil type, light, temperature, humidity, plant types and other soil microorganisms. For example, microorganisms in EM may have positive or negative effects on the growth of predator populations. The numbers of predators in the field could influence the reproduction of springtails because the predators may feed on eggs and kill juvenile and adult springtails whereas the population of other soil microorganisms may

increase due to the preferred fungi in EM and, in turn, help increase the organic matter and preferred food of springtails. This might lead to an increase in the springtail population in nature.

ACKNOWLEDGEMENTS

This research was supported and facilitated by the Department of Biology, Faculty of Science, Mahidol University, Bangkok, Thailand. Financial support was provided by the Development and Promotion of Science and Technology Talents Project. The authors would like to thank Boongeua Vajarasathira, Department of Zoology, Faculty of Science, Kasetsart University, Bangkok, Thailand and Charnwit Kositanont, Department of Microbiology, Faculty of Science, Chulalongkorn University, Bangkok, Thailand for constructive advice and valuable suggestions.

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